Renal function after solid organ transplantation

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Chapter 2

Creatinine-based estimation of rate of long-term renal function loss in lung transplant recipients. Which method is preferable?

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Abstract

Progressive renal function loss during long-term follow up is common after lung transplantation and close monitoring is warranted. Since changes in creatinine generation and excretion may occur after lung transplantation, the reliability of creatinine-based methods of renal function assessment to serial measurements of glomerular filtration rate (GFR) were compared in this population.

Renal function with serial measurements of GFR by iothalamate clearance every 6 months after transplantation was studied in a cohort of 40 lung transplant recipients with at least 24 months of follow up, transplanted between November 1990 and October 1995 in this centre. The correlation between the rate of renal function loss calculated from the slope of GFR and the following creatinine-based indices: the reciprocal of serum creatinine ($1/S_{creatinine}$), Cockcroft clearance and Levey estimation were analysed. The absolute difference between GFR and Cockcroft clearance and Levey estimation pre- and at several points post-transplantation was also studied.

The slopes of $1/S_{creatinine}$ (r=0.85), Cockcroft clearance (r=0.86), and the Levey estimation (r=0.84) correlated significantly with the slope of GFR as measured by iothalamate clearance. However, all creatinine-based slopes underestimate the rate of GFR loss. Cockcroft clearance and the reciprocal value of $S_{creatinine}$ do not detect small GFR losses. During long-term follow up a time-dependent discrepancy between Cockcroft clearance, Levey estimation and GFR was observed which may partially explain the observations for this population.

Creatinine-based slopes correlate with GFR slopes after lung transplantation, but consistently underestimate the rate of GFR decline. The Levey estimation is the most sensitive method to detect small GFR losses and may be preferable when no GFR measurement is available. In special conditions when an accurate renal function assessment is needed true GFR may be necessary.

Introduction

As progressive renal function loss is common after heart- and lung transplantation[1,2] close monitoring of renal function is warranted. The standard used to monitor chronic renal function loss is serial measurement of glomerular filtration rate (GFR)[3]. In clinical practice, however, this is not always feasible. It would be convenient to rely on renal function estimates based on $S_{creatinine}$ only. $S_{creatinine}$ depends not only on GFR but also on muscle mass, and renal tubular creatinine secretion induces additional bias. To account for the resulting error several correction formulas such as the Cockcroft-Gault formula[4] and the recent estimation developed by Levey[5], are available. These formulas were empirically derived, and validated mostly in cross-sectional studies in renal patients.

Whether the assumptions on body mass composition and tubular creatinine secretion that underlie these estimations similarly apply to lung transplant recipients is unknown. Specific alterations of tubular creatinine secretion, due to drug use[6] or the underlying disease[7], are likely to occur in this population. Their reliability for longitudinal renal follow up, moreover, depends on the assumptions that body mass composition and creatinine secretion do not change over time[8,9,10].

The reliability of $S_{creatinine}$-based estimates of long-term renal loss by comparing these estimates with serial GFR measurements by iothalamate clearance was investigated in lung transplant recipients. For long-term renal monitoring, calculation of the slope of renal function loss from repeated measurements has the advantage of circumvening the bias of short-term fluctuations and providing an estimate of renal prognosis. Thus, the slopes of measured GFR over time was compared with the slopes derived from $1/S_{creatinine}$, the Cockcroft-Gault formula and the Levey formula, respectively, in a closely monitored cohort of lung transplant recipients with at least 24 months of follow up. For all three $S_{creatinine}$-based methods the agreement as to the rate of renal function loss was assessed, as well as the sensitivity for detection of small renal function loss. In addition, the Cockcroft-Gault clearance, the GFR estimated from the Levey equation and the GFR measured by iothalamate clearance were compared to see if the relations remained constant over time.

Patients and Methods

Patients

Consecutive patients, receiving a bi- or unilateral lung transplant between November 1990 and October 1995, with at least 24 months of follow up and at least 4 GFR measurements from 6 months onwards were included in this study. The latter criterion required for an accurate calculation of the slopes of GFR, $1/S_{creatinine}$, Cockcroft-Gault estimated creatinine

clearance (Cockcroft clearance) and GFR estimated according to the Levey equation (Levey estimation) over time. Of seventy patients transplanted in this period, 40 patients fulfilled these criteria. Thirty patients were excluded due to patient death (n=20) or re-transplantation (n=2) within 24 months after transplantation or for lacking GFR measurements (n=8).

Methods

Immunosuppressive treatment is described in detail elsewhere[1]. Briefly, induction therapy was given for 7 days with rabbit anti-human thymocyte globulin (RIVM, Bilthoven, The Netherlands). Maintenance immunosuppression consisted of a triple-drug regimen with cyclosporin A (CyA) (Sandimmune or Neoral), azathioprine, and corticosteroids, starting during transplantation. The target CyA serum trough level was initially 400 µg/l tapering to 150 µg/l, which was considered the lowest acceptable level. All patients received post-operative antibiotic prophylaxis with ceftazidime. Antibiotics were changed, if necessary, in accordance with sputum or other cultures. Aminoglycosides were used when necessary, under close monitoring of serum levels. Prophylaxis for herpes infections consisted of oral aciclovir (4x200 mg) during the first 6 months, and prophylaxis for Pneumocystis carinii consisted of oral co-trimoxazole 800/160 mg every other day for the lifetime of the patient. Renal function studies were performed in all patients during the pre-transplant workup and were repeated every 6 months after transplantation. $S_{creatinine}$ was measured (SMA(C) autoanalyser; Technicon Instruments, Tarrytown, NY, USA) on the same day as GFR. From these values $1/S_{creatinine}$ was calculated and creatinine clearance was estimated according to the formula of Cockcroft and Gault (Cockcroft clearance)[4]:

$$\text{estimated creatinine clearance (ml/min)} = \frac{(140 - \text{age}) \times \text{bodyweight (kg)}}{S_{creatinine\ (mg/dl)} \times 72}$$

(for women the outcome is multiplied by 0.85)

$S_{creatinine}$ was also used to predict GFR with the MDRD (Modification of Diet in Renal Disease) study equation 7 (Levey estimation)[5]:

$$\text{estimated GFR (ml/min per 1.73 m}^2) = 170 \times S_{Cr(mg/dl)}^{-0.999} \times \text{age}^{-0.176} \times S_{U(mg/dl)}^{-0.170} \times S_{Alb(g/dl)}^{0.318}$$

(for women the outcome is multiplied by 0.762 and for black persons with 1.180)

GFR was measured as the urinary clearance of constantly infused ^{125}I-iothalamate. Simultaneous measurement of ^{131}I-hippuran clearance allowed to correct for errors induced by incomplete bladder emptying as described previously. GFR measurements performed in

this way have a variation coefficient of only 2.2 %, which allows accurate follow up of renal function[3]. The measured GFR and the estimated Cockcroft clearance were corrected to 1.73 m^2 of body surface.

Statistical analysis

Data are presented as mean with standard deviation and where indicated with total range, unless stated otherwise. Differences in continuous variables between and within groups were tested with the unpaired and paired Student's t-test, respectively. The rate of long-term renal function loss was defined as the individual slope over time of the change in GFR, of $l/S_{creatinine}$, of the Cockcroft clearance or of the Levey estimation, and calculated by least squares linear regression. Slopes were calculated, using all available data points, as of 6 months post-transplant because a biphasic course of renal function in patients after lung transplantation was found previously[1]. Correlation between the individual slopes over time of GFR and $l/S_{creatinine}$, of the Cockcroft formula and of the Levey estimation were tested with least squares linear regression analysis. Regression coefficients are given with the 95% confidence interval (CI)[11]. To assess whether the relation between GFR, Cockcroft clearance and the Levey estimation is constant over time, the absolute difference between GFR and Cockcroft clearance and Levey estimation, respectively, was also studied pre- and at several time points post-transplantation.

Results

Patient characteristics are shown in *table 1 (pag. 28)* . Most patients were transplanted for emphysema, while a minority had pulmonary hypertension, cystic fibrosis or lung fibrosis as the cause of respiratory failure. Renal function loss is most rapid in the first 6 months post-transplant as can be seen in *figure 1 (pag. 29)*. After 6 months a more gradual fall is evident later on in most patients. The mean individual slope of GFR from 6 months after transplantation onwards was -7.3 ± 6.6 $ml.min^{-1}.yr^{-1}$ per 1.73 m^2 (range, –22.8 to +6.1). Before transplantation $S_{creatinine}$ was 0.079 ± 0.018 mmol/l (range 0.050 to 0.133). $S_{creatinine}$ increased to 0.120 ± 0.028 mmol/l (range 0.056 to 0.187) at 6 months, 0.137 ± 0.030 mmol/l (range 0.067 to 0.202) at 12 months, 0.148 ± 0.040 mmol/l (range 0.092 to 0.235) at 24 months, 0.167 ± 0.061 mmol/l (range 0.086 to 0.296) at 36 months, and 0.175 ± 0.061 mmol/l (range 0.097 to 0.309) at 48 months post-transplant.

The mean slope of $1/S_{creatinine}$ from 6 months after transplantation onwards was -0.53 ± 0.79 $l.mmol^{-1}.yr^{-1}$ (range, –2.58 to +1.32). Individual slopes of $1/S_{creatinine}$ correlated significantly with the individual slopes of GFR (r=0.86). The intercept with the horizontal axis was –2.2 $ml.min^{-1}.yr^{-1}$. 1.73 m^{-2} (95% CI, –5.1 to –0.2) *(figure 2A, pag. 30)*.

Table 1 Patient characteristics in the group of 40 lung transplant recipients.

Number (n)	40
Mean age (yr)	43 ± 10
Female/male	17/23
Pulmonary diagnosis	
• Pulmonary hypertension	6
• Emphysema	25
• Cystic fibrosis	6
• Lung fibrosis	3
Pre-transplantation*	
• Serum creatinine (mmol.l^{-1})	0.078 ± 0.018 (0.050 to 0.133)
• $1/S_{creatinine}$ (l.$mmol^{-1}$)	13.1 ± 3.0 (7.5 to 20)
• GFR (ml.min^{-1}.1.73m^{-2})	100 ± 22 (65 to 171)
• Cockcroft clearance (ml.min^{-1}.1.73m^{-2})	91 ± 21 * (64 to 152)
• Levey formula (ml.min^{-1}.1.73m^{-2})	93 ± 22 * (60 to 156)
Follow up (months) **	47 ± 15 (24 to 78)

* $p < 0.001$ as compared to GFR
** mean ± standard deviation (total range)

The mean slope of the Cockcroft clearance from 6 months after transplantation onwards was -4.1 ± 5.7 ml.min^{-1}.yr^{-1}.1.73 m^{-2} (range, –18.6 to +11.3). A significant correlation was present between the individual slopes of the Cockcroft clearance and GFR (r=0.87). The intercept with the horizontal axis was –1.8 ml.min^{-1}.yr^{-1} per 1.73 m^2 (95% CI, –4.5 to 0.3). The regression line is not parallel to the line of identity, with a regression coefficient of 0.75 (95% CI, 0.61 to 0.89), indicating a mean underestimation of 25% of the rate of renal function loss as measured by GFR slope over the whole range *(figure 2B, pag. 30)*.
The mean slope of the Levey estimation from 6 months after transplantation onwards was –4.3 ± 5.1 ml.min^{-1}.yr^{-1}.1.73 m^{-2} (range, –16.1 to +10.0) with a significant correlation between the individual slopes of the Levey estimation and GFR as well (r=0.84). The intercept with the horizontal axis was –0.6 ml.min^{-1}.yr^{-1}.1.73 m^{-2} (95% CI, –3.5 to +1.2).

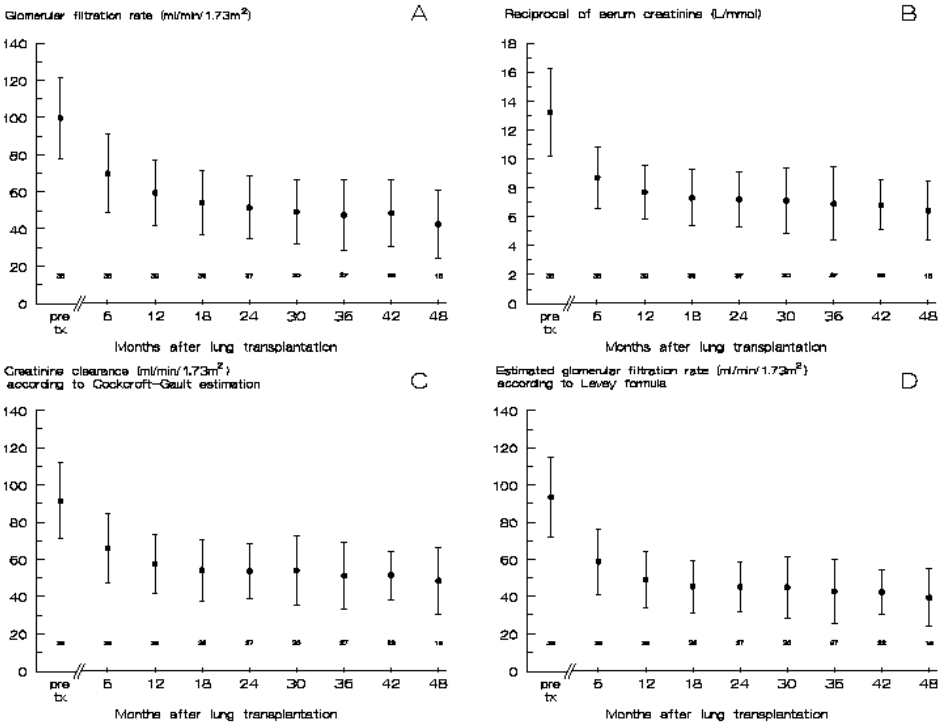

Figure 1 Mean glomerular filtration rate GFR (ml/min/1.73m²) (A), reciprocal value of $S_{creatinine}$ (l/mmol) (B), creatinine clearance estimated according to the Cockcroft-Gault formula (ml/min/1.73m²) (C), and GFR estimated according to the Levey equation (ml/min/1.73m²) (D) pre- and post-transplantation in 40 lung transplant recipients. The error bars indicate ± 1 standard deviation. The numbers above the horizontal axis indicate the number of patients with GFR measurements at the time point.

The regression line is not parallel to the line of identity, with a regression coefficient of 0.65 (95% CI, 0.51 to 0.79), indicating a mean underestimation of 35% of the rate of renal function loss as measured by GFR slope over the whole range *(figure 2C, pag. 30)*.

The intercept with the horizontal axis of the regression lines of both the slopes of $1/S_{creatinine}$ *(figure 2A, pag. 30)* and Cockcroft clearance *(figure 2B, pag. 30)* indicates that these methods have a higher threshold for detecting GFR decline than iothalamate clearance. The regression line of the slopes of the Levey estimation intercepts the horizontal axis more closely to the origin *(figure 2C, pag. 30)*, indicating that this method is somewhat more sensitive than the other creatinine-based estimates in the detection of small losses in GFR. Both the correlations between 3 creatinine-based slopes and the slopes of measured GFR

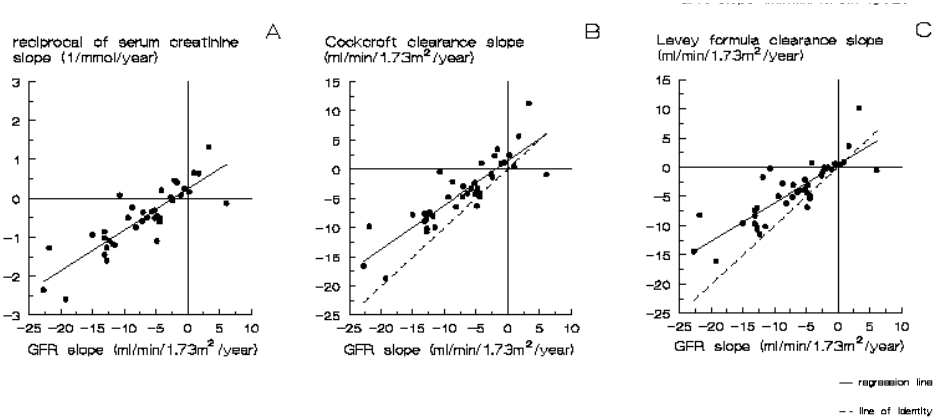

Figure 2 Correlation between slopes over time of measured glomerular filtration rate (GFR), reciprocal value of $S_{creatinine}$ (A), slopes of Cockcroft-Gault estimated creatinine clearance (B), and slopes of the GFR estimated according to the Levey equation (C) in 40 lung transplant recipients with at least 4 measurements during follow up. The solid line indicates the calculated regression line, the dotted line indicates the line of identity (B and C).

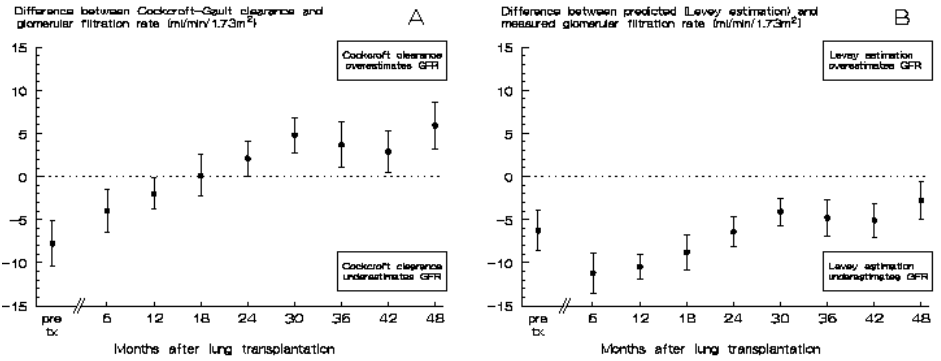

Figure 3 Mean difference (ml/min/1.73m^2) between Cockcroft-Gault estimated creatinine clearance (A), estimated GFR according to the Levey equation (B) and measured glomerular filtration rate (ml/min/1.73m^2) in 40 lung transplant recipients. Error bars indicate the standard error of the mean differences.

and the intercepts with the horizontal axis were not different for male and female lung transplant recipients (data not shown).
The mean of the differences between Cockcroft clearance, GFR estimated from the Levey equation, and GFR measured by iothalamate clearance before and at several time points after transplantation is shown in *figure 3*. Remarkably, pre-transplantation and during the first year after lung transplantation Cockcroft clearance underestimates GFR, but during further follow up it overestimates GFR *(figure 3A)*. The GFR estimated from the Levey equation underestimates true GFR both before and after transplantation. The magnitude of the underestimation tends to decrease over time *(figure 3B)*. These results are independent from pulmonary diagnosis and gender (data not shown).

Discussion

The population of patients receiving a lung transplant is relatively small. In order to be able to properly analyse the course of renal function in this population, renal function is monitored by frequent ^{125}I-iothalamate clearance measurements in this centre. Prior analysis has demonstrated that this allows accurate calculation of the rate of renal function loss in individual patients, and alleviates the need for large populations to obtain sufficient statistical power to detect differences[3].
Serial measurements of GFR showed a considerable and progressive renal function loss after transplantation in the patients. Overall, the slopes of the reciprocal value of $S_{creatinine}$, of Cockcroft clearance and of the Levey estimation correlate reasonably well with the renal function slopes measured by serial iothalamate clearances. However, the creatinine-based slopes underestimate the rate of GFR decline. First, the slopes of the reciprocal of $S_{creatinine}$ and of the Cockcroft clearance have a higher threshold to detect renal function decline than GFR measured by iothalamate clearance. A zero slope as calculated by these methods suggests a stable renal function, but in fact corresponds to a GFR loss of approximately – 2 $ml.min^{-1}.yr^{-1}.1.73\ m^{-2}$. Of the creatinine-based methods, the Levey method appears to have the lowest threshold to detect GFR decline. Second, and more important from a clinical perspective, the creatinine-based methods also underestimate the rate of GFR decline when renal function loss is more pronounced, i.e. by some 25% with the Cockcroft formula and by 35% with the Levey equation. Furthermore, the large interindividual variability, as illustrated by the data in *figure 2*, leads to a wide confidence interval in the prediction of the GFR slope based on creatinine-based methods. Therefore, available creatinine-based methods to monitor renal function decline in this population suffer from considerable, and clinically relevant flaws.
We found that the relation of iothalamate clearance with the Cockcroft clearance and the Levey-estimate of GFR was not constant over time. An overestimation of GFR by the

Cockcroft clearance occurred gradually with longer follow up. Likewise, the underestimation of GFR by the Levey equation gradually diminished over time. These changes over time could well explain the significantly lower slopes as compared to serial iothalamate clearances of renal function decline calculated by these creatinine-based methods. Decreased creatinine generation, increased tubular creatinine secretion, or both over time could both cause the observed changes during long-term follow up[10,12]. Decreased creatinine generation over time could be due to loss of muscle mass as a result of long-term use of corticosteroids. As we have no measurements of lean body mass, or of creatinine excretion in our patients, however, we cannot substantiate this assumption. Increased secretion of creatinine has been reported with specific mutations (DF508) underlying cystic fibrosis[7], but in our population the discrepancy was present irrespective of diagnosis. The use of cyclosporin A and co-trimoxazole in our population would be expected to decrease rather than increase tubular creatinine secretion. These medications were unchanged during follow up and thus do not explain our observation. The relative contribution of tubular secretion in renal creatinine clearance is known to increase with deteriorating renal function[10], which could explain the discrepancy over time. If so, one would expect similar observations in other populations as well, but no systematical time-dependent difference between creatinine-based estimates of GFR and iothalamate clearance has been found until now[12,13]. Finally, the accuracy of the estimation of creatinine clearance or GFR from $S_{creatinine}$ depends on the assay used to measure $S_{creatinine}$[14], which may explain differences between studies but not within one single centre.

The Levey- and Cockcroft-Gault estimations of renal function use anthropometric and other data to estimate 24-hour creatinine generation without collecting 24-hour urine, in order to improve the relation between individual $S_{creatinine}$ and renal function. Even without measured 24-hour urine creatinine excretion the Levey estimation is claimed to provide a more accurate estimation of GFR than does measured creatinine clearance[5]. Both the Cockcroft-Gault formula and the Levey equation have been derived from cross-sectional data in populations that are grossly comparable to our lung transplant recipients with respect to age, gender and renal function. The Cockcroft-Gault formula modelled measured creatinine clearance from $S_{creatinine}$ and 24-hour urine creatinine excretion in a large data set obtained in males and females with different levels of renal function and body composition as a function of anthropometric characteristics and $S_{creatinine}$ only. The Levey estimation modelled GFR as measured by iothalamate clearance to a combination of anthropometric and laboratory data in male and female patients with renal disease and moderate degrees of renal function impairment. Both equations include a multiplication factor for females to correct the results for the gender dependent muscle mass differences. Although our groups are small, the data do not suggest that the discrepancies found between measured and estimated GFR are caused by a difference in only one of the sexes. For a proper interpretation of our findings it

is important to realise that the population of lung transplant recipients is not only clearly different from the populations studied by Cockcroft, Gault and Levey, but that the latter estimations were calculated from cross-sectional data only. Consequently, the models derived from these populations are not subject to bias due to changes in anthropometric characteristics and creatinine generation. In our population, however, a major change in clinical condition is elicited by the lung transplantation. Apparently, this results in an altered relationship between true and estimated GFR, before and after transplantation. Moreover, we show that during long-term follow up, with the patients in a relatively stable clinical condition, the relationship between estimated and true GFR is not constant over time. The maximum follow up in our study was 48 months and it is unclear whether this time dependent effect on the relation between GFR, Cockcroft clearance and Levey formula has already levelled off at that time.

What would be the implications of our findings? Clearly, monitoring of renal function by $S_{creatinine}$ only elicits a systematical underestimation of the rate of renal function loss in lung transplant recipients. Measurement of $S_{creatinine}$ is a simple method for estimating renal function, but our findings demonstrate its limited reliability as a tool to monitor long-term renal function loss in this population. From our data we cannot establish whether calculation of creatinine clearance from 24-hour urine might provide a better estimate, as this would only correct for bias by decreased creatinine generation but not for bias by increased tubular creatinine secretion. In clinical practice, accurate collection of 24-hour urine requires patient compliance and introduces additional sources of error. In general, $S_{creatinine}$-based methods to monitor renal function during long-term follow up can only be reliable if the assumptions on the relation between $S_{creatinine}$ and GFR are valid and do not change over time in the population studied. Our study shows that these criteria are not met in the population of lung transplant recipients. Despite its costs serial GFR measurement is therefore preferable in observational and intervention studies on renal function in these populations. Even in clinical care, under specific circumstances GFR measurement may be necessary for correct evaluation of renal function. For instance when renal function is a determinant in acceptance for lung transplantation or has major impact in the treatment during follow up.

In conclusion, although slopes calculated from $S_{creatinine}$-based estimates of renal function correlate with slopes of measured GFR decline after lung transplantation, they consistently underestimate the rate of GFR decline. This inaccuracy will have to be accounted for in this specific population when using creatinine-based monitoring.

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